PATENT
Customer No. 22,852
Attorney Docket No. **06478.1473**

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	
Mirna RAPP	Group Art Unit: 1656
Application No.: 09/869,031	Examiner: Holly G. Schnizer
§371 Filing date: October 16, 2001	Confirmation No.: 7920
For: **FIBRIN-BASED GLUE GRANULATE AND CORRESPONDING PRODUCTION**	

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

RESPONSE TO THE OFFICE COMMUNICATION OF APRIL 18, 2006, AND SUGGESTION OF INTERFERENCE PURSUANT TO 37 C.F.R. § 41.202

In response to the Office Communication dated April 18, 2006, the period for response being extended by the accompanying five-month Petition for Extension of Time and fee, and pursuant to 37 C.F.R. § 41.202, Applicant submits this Suggestion of Interference and respectfully requests that the Office declare an interference between the present application and U.S. Patent No. 6,596,318.

Applicant is filing an Amendment concurrently with this paper canceling claims in the instant application not drawn to the interfering subject matter defined below.

Applicant presents below the information requested under 37 C.F.R. § 41.202 under headings that mirror the subsections of § 41.202, in order to facilitate the Office's consideration of this information.

I. Statement on the Substance of Interview

Applicant thanks Examiner Holly G. Schnizer for indicating the nature of the extensions of time available to respond to the Office Communication of April 18, 2006, during a telephone interview with the undersigned on May 17, 2006. This was the only topic addressed during the interview and Applicant agrees with the Examiner's Interview Summary recording the telephone interview. Applicant presents this statement in compliance with 37 C.F.R. § 1.133.

II. Identification of the patent with which applicant seeks an interference

Applicant seeks an interference between instant U.S. Application No. 09/869,031 (the '031 application), and U.S. Patent No. 6,596,318 (the '318 patent), filed on April 26, 2001, and issued to A. Prasch and B. Luy on July 22, 2003.

III. Identification of interfering subject matter

The interfering subject matter of this suggested interference is drawn generally to fibrin tissue adhesive granulates comprising thrombin, Factor XIII, and fibrinogen, wherein the granulates have a particle size in the range from 50 µm to 1000 µm. More specifically, claims 25-28, 37, 43-49, 52-57, 59-65, and 67-73 of the '031 application and claims 1-19 of the '318 patent define interfering subject matter within the meaning of 37 C.F.R. § 41.203(a).

An interference is appropriate between an application and an unexpired patent of different parties if the subject matter claimed by one party would have, if prior art, anticipated or rendered obvious the subject matter of a claim of the opposing party and vice versa. 37 C.F.R. § 41.203(a). That is, an interference is appropriate when the application and patent contain claims to the same patentable invention. The Board uses a two-way test to determine the presence of interfering subject matter. *See Eli Lilly & Co. v. Board of Regents of the University of Washington,* 334 F.3d 1264, 67 U.S.P.Q.2d 1161 (Fed. Cir. 2003).

With respect to claims 59-65 and 67-73, the two-way test is satisfied here at least because these claims were copied from claims in the '318 patent. Claims 59-65 and 67-73 were modified only to account for the minor differences in the manner in which the interfering subject matter was disclosed in the instant application and in the '318 patent. A difference worth noting between the instant claims and the claims in the '318 patent is the range of particle sizes for the fibrin tissue adhesive granulates. The range for the granulates in the '031 application is from 50 to 1000 µm, whereas the range for the granulates in the '318 patent is from 20 to 1000 µm. This difference, however, does not preclude a finding that the instant application and the '318 patent define the same patentable subject matter because there is substantial overlap in the ranges and an otherwise identical claim to a fibrin tissue adhesive granulate of one particle size range would be an obvious variation of a claim to a fibrin tissue adhesive granulate of the other particle size range.

Additionally, claims 25-28, 37, 43-49, and 52-57 of the '031 application also define subject matter interfering with the claims of the '318 patent. The table in

Appendix A compares independent claims 25, 43, 59, 63, 64, and 70 of the '031 application with independent claims 1, 5, 6, and 16 of the '318 patent. Section V below explains in detail how the claims from the '031 application and the '318 patent interfere and how they correspond to the proposed counts.

IV. Proposed counts

In accordance with 37 C.F.R. § 41.202(a)(2), Applicant proposes the following two counts to define the interfering subject matter of this suggested interference.

Count 1

> A fibrin tissue adhesive formulation containing a mixture of thrombin, and fibrinogen with factor XIII in flowable solid granules, said mixture prepared by:
>
> (a) providing solutions or suspensions of the thrombin, and the fibrinogen with factor XIII;
>
> (b) drying the solutions in a fluidized bed apparatus; and
>
> (c) forming the flowable solid granules with a particle size of approximately 50-1000 µm.
>
> Or
>
> A fibrin tissue adhesive granulate comprising granulate pellets with a particle size in the range from approximately 50 µm to approximately 1000 µm,
> wherein said granulate pellets comprise thrombin, Factor XIII, fibrinogen, and a calcium salt.

Count 1 incorporates in the alternative the language of claims 59 or 25 of the '031 application, respectively.

Count 2

A process for producing a fibrin tissue adhesive formulation containing a mixture of thrombin, and fibrinogen with factor XIII in flowable solid granules, which comprises

(a) providing solutions or suspensions of the thrombin, and the fibrinogen with factor XIII;

(b) drying the solutions in a fluidized bed apparatus; and

(c) forming the flowable solid granules, said granules having a particle size of 50-1000 µm.

Or

A method for the preparation of a fibrin tissue adhesive granulate comprising,

suspending the components of the fibrin adhesive in an organic solvent, and

spray-drying said suspension

to a granulate of particle size in the range from 50 µm to 1000 µm;

wherein the fibrin tissue adhesive granulate comprises thrombin, Factor XIII, fibrinogen, and a calcium salt.

Count 2 incorporates in the alternative the language of claims 70 or 43 of the '031 application, respectively.

V. Claims corresponding to the counts

A claim should be designated as corresponding to the count if, considering the count as prior art, the claim would be unpatentable over the count under 35 U.S.C. § 102 or § 103. 37 C.F.R. § 41.207(b)(2). In addition to the explanation below, Applicant also provides in Appendix B a table comparing each count with at least one claim from each party as required by 37 C.F.R. § 41.202(a)(3).

a. Count 1

i. Claims from the '031 application

Claims 25-28, 37, 52-57, 59-65, and 67-69 from the '031 application correspond to proposed Count 1. Independent claims 25 and 59 correspond to Count 1 because Count 1 was drafted in the alternative incorporating the language from claims 25 or 59. Claims 26 and 67 recite a fibrin tissue adhesive granulate in accordance with claims 25 or 59 respectively, wherein the granulate pellets have a particle size in the range from 100 µm to 200 µm. Claims 26 and 67 would have been obvious if Count 1 was considered prior art because these claims are drawn to a range of particles sizes that overlap with the range in the count and which one of ordinary skill in the art would have found when optimizing the particle size of the fibrin tissue adhesive granulate disclosed in Count 1. *See, e.g.*, the '318 patent at col. 4, lines 25-31. Therefore, claims 26 and 67 correspond to claim 1.

Claims 27 and 28 are drawn to fibrin tissue adhesive granulates according to claims 25 and 26 respectively, further comprising, *inter alia*, one or more substances that promote wound healing. Given that one of the utilities of fibrin tissue adhesives is to promote wound healing (specification of the '031 application at p. 2, ¶ 3), claims 27 and 28 would have been obvious in light of Count 1 because one of ordinary skill in the art would have been led to add one or more substances that promote wound healing to a composition already used in promoting wound healing. For analogous reasons, claims 52, 53, and 57, which are drawn to methods of preparing formulations comprising a fibrin tissue adhesive granulate as claimed in claim 1 and one or more active substances, would also have been obvious in light of Count 1. Therefore,

because claims 27, 28, 52, 53, and 57 would have been obvious in light of Count 1, if count 1 was considered prior art, they correspond to Count 1.

Claims 54-56 are drawn to methods of achieving hemostasis, healing a wound, and effecting tissue therapy, respectively, comprising applying a fibrin tissue adhesive granulate to an area in need thereof. These claims would have been obvious in light of Count 1 because one of ordinary skill in the art would have been led to use the fibrin tissue adhesive granulate according to Count 1 in the method claimed based on the fact that one of the utilities of fibrin tissue adhesives is to promote wound healing. Specification of the '031 application at p. 2, ¶ 3.

Claim 60 is drawn to a fibrin tissue adhesive granulate wherein the thrombin and fibrinogen granules have been separately dried. This claim would have been obvious to one of ordinary skill in the art if Count 1 was prior art because preparation of separate formulations of thrombin and fibrinogen is a method well known in the art to store fibrin tissue adhesives. *See, e.g.*, specification of the '031 application at p. 3, lines 3-6.

Claim 61 recites a fibrin tissue adhesive granulate prepared using a support medium in general as a carrier for the granulates, and claim 62 recites specific substances that can be used as the support medium. Claim 63 recites a fibrin tissue adhesive granulate incorporating fibrinogen in an inner core and thrombin in an outer layer thereon. Claim 64 recites fibrin tissue adhesive granulates comprising a carrier core, a fibrinogen layer on the core and an outer thrombin layer. Claim 68 is drawn to fibrin tissue adhesive granulate covered with an outer barrier layer. These claims would have been obvious to one of ordinary skill in the art in light of Count 1 because they represent known and obvious variations of the methods known in the art for the

preparation of granulates (optional use of a support medium as carrier, applying further layers of active ingredients on top of the carrier, etc.) For example, for a two-component granulate, some of the available options are to have two separate granulates, one for each component, or to have a mixed granulate, with one component forming the core, and the second component being spray-dried onto that core. *See also* U.S. Patent No. 5,840,329, which describes drug delivery systems wherein particles comprising one or more active ingredients, in a core or in further layers, are used in delivery systems. See, e.g., cols. 1-2.

Claim 65 recites a fibrin tissue adhesive granulate with a particular ratio of thrombin to fibrinogen, which would have been obvious to one of ordinary skill in the art over Count 1 because this ratio is already known and used in the preparation of fibrin adhesives in general. *See, e.g.*, the '318 patent at col. 2, lines 62-64.

Claim 69 recites a fibrin tissue adhesive granulate further comprising a calcium salt. Claim 69 would have obvious if Count 1 was considered prior art because calcium salts are a common component of fibrin adhesives. *See, e.g.*, the '031 application at p. 2, lines 9-12.

ii. Claims from the '318 patent

Claims 1-15 from the '318 patent correspond to Count 1. As mentioned previously, claim 1 of the '318 patent is virtually identical to claim 59 of the '031 application, which forms one of the bases of Count 1, except for the particle size range recited therein. Claim 1 recites a particle size range from 20-1000 µm, whereas Count 1 recites a particle range from 50-1000 µm. If Court 1 is considered prior art, then either of the end points of the particle size range, *i.e.*, 50 and 1000 µm, would

anticipate claim 1 of the '318 patent. For at least this reason, claim 1 of the '318 patent corresponds to Count 1.

Claims 2-6 and 9, 12, 13, and 15 of the '318 patent are drawn to substantially the same subject matter as claims 60-65, and 67-69 of the '031 application respectively, given that these claims in the '031 application were copied from the corresponding claims in the '318 patent. Therefore, claims 2-6 and 9, 12, 13, and 15 of the '318 patent would also be obvious over Count 1 for the same reasons explained above for claims 60-65, and 67-69 of the '031 application. For example, the use of granulates wherein the thrombin and fibrinogen granules have been separately dried in claim 2 would have been obvious to one of ordinary skill in the art if Count 1 was prior art because preparation of separate formulations of thrombin and fibrinogen is a method well known in the art to store fibrin tissue adhesives. *See, e.g.*, specification of the '031 application at p. 3, lines 3-6. The use of core carriers (claims 3 and 4), and separate layers comprising thrombin or fibrinogen (claims 5 and 6) would have been obvious if claim 1 was considered prior art because such techniques for providing active ingredients within particles are well within the knowledge of one of ordinary skill in the art as explained before. *See, e.g.*, U.S. Patent No. 5,840,329, at cols. 1-2.

Claim 7 recites a fibrin tissue adhesive formulation, wherein a barrier layer is present between the fibrinogen layer and the outer thrombin layer. Claim 7 would have been obvious if Count 1 was considered prior art because one of ordinary skill in the art would have been motivated to use a barrier layer to separate thrombin from fibrinogen before the fibrin tissue adhesive is applied to a desired area, in order to minimize premature clotting, which occurs when thrombin and fibrinogen are placed in contact

with each other in solution. *See*, *e.g.*, the '318 patent at col. 2, lines 16-20; col. 4, lines 51-57. Additionally, the use of barrier layers in particles comprising one or more active ingredient is known in the art in order to keep those components separate. *See*, *e.g.*, U.S. Patent No. 5,840,329, at col. 2, lines 18-31; and col. 5, lines 43-53. Claim 8 recites a fibrin tissue adhesive formulation wherein the barrier layer is produced by drying solutions of low-molecular polyvinylpyrrolidone; cellulose derivatives; or carbohydrates. Claim 8 would have been obvious if Count 1 was considered prior art because those components are well known in the art as components of barrier layers (*see*, *e.g.*, the '318 patent at col. 4, lines 55-60; U.S. Patent No. 5,840,329, at col. 8, lines 12-33), and one of ordinary skill in the art would have been motivated use a barrier layer to separate fibrinogen and thrombin as discussed above. Claim 13 recites a fibrin tissue adhesive formulation, wherein the granules are provided with an outer barrier layer. Claim 13 would have been obvious if Count 1 was considered prior art because one of ordinary skill in the art would have been motivated to use an outer layer to facilitate rapid hydration of the components of the particle. *See*, *e.g.*, U.S. Patent No. 6,261,601 at col. 10, lines 48-56.

The specific thrombin to fibrinogen ratios recited in claims 9 and 10, are either already known or would have been obvious to one of ordinary skill in the art through routine optimization if count 1 was prior art. *See*, *e.g.*, the '318 patent at col. 2, lines 62-64, indicating that "[s]uitable ratios [of thrombin to fibrinogen] are known according to the state of the art;" see also the '318 patent at col. 4, lines 27-31.

Claims 11 and 12 of the '318 patent are drawn to fibrin tissue adhesive granulates reciting a particular ratio of thrombin to fibrinogen or a particular particle size

range. These ratios and ranges overlap with, and are obvious variations of the original values recited in the count. Alternatively, the ratios and ranges would have been found by one of ordinary skill in the art when optimizing these parameters for a particular application. *See, e.g.*, the '318 patent at col. 4, lines 25-27. Thus, claims 11 and 12 would also be obvious to one of ordinary skill in the art if Count 1 was considered prior art.

Claim 14 of the '318 patent recites a fibrin tissue adhesive granulate wherein the thrombin and fibrinogen are produced recombinantly. This limitation does not make this claim patentable over Count 1 because it would have been obvious to use any source of thrombin and fibrinogen in the preparation of the fibrin tissue adhesive granulates of Count 1, regardless of whether they were produced recombinantly or not. *See, e.g.*, U.S. Patent No. 6,037,457 (filed on January 1997), indicating that non-recombinant fibrinogen has the disadvantage of potential contamination by impurities and pathogens, which make recombinant fibrinogen preferable. Col 4, lines 4-7.

Claim 15 is drawn to a fibrin tissue adhesive granulate wherein the solution of suspension contains a calcium salt. Claim 15 would have obvious if Count 1 was prior art because calcium salts are a common component of fibrin adhesives. *See, e.g.*, the '031 application at p. 2, lines 9-12.

In summary, claims 1-15 of the '318 patent would have been obvious over Count 1, and therefore correspond to Count 1.

b. Count 2

i. Claims from the '031 application

Claims 43-49 and 70-73 from the '031 application correspond to proposed Count 2. Claims 43 and 70 correspond to Count 2 because Count 2 was drafted in the alternative incorporating the language from claims 43 and 70.

Claim 44 recites a method in accordance with claim 43, wherein the granulates have a particle size in the range from approximately 100 µm to approximately 200 µm. Claim 44 would have been obvious in light of Count 2, if Count 2 was considered prior art, because the claim is drawn to a range of particles sizes that overlap with the range in the count and which one of ordinary skill in the art would have found when optimizing the particle size of the method disclosed in Count 2. *See, e.g.*, the '318 patent at col. 4, lines 25-27.

Claims 45, 46, and 73 recite methods for the preparation of a fibrin tissue adhesive granulate wherein the suspension comprising fibrin adhesive components is spray-dried onto a support medium. These claims would have been obvious to one of ordinary skill in the art in light of Count 2 because they represent methods of preparation of granulates already known in the art of spray drying. *See, e.g.*, the '318 patent at col. 4, lines 43-45.

Claims 47 and 72 recite a method for the preparation of a fibrin tissue adhesive granulate wherein an organic solvent comprising thrombin is sprayed onto a fibrinogen granulate. Claims 49 and 71 recite a method wherein separate thrombin and fibrinogen granulates are prepared. These claims would have been obvious to one of ordinary skill in the art in view of Count 2 because they represent obvious variations of the steps

recited in the count. For example, for a two-component granulate, the available options are to have two separate granulates, one for each component, or to have a mixed granulate, with one component forming the core, and the second component being spray-dried onto that core. Moreover, the preparation of formulations wherein thrombin and fibrinogen are not in solution with each other is a method well known in the art to store fibrin tissue adhesives. *See*, *e.g.*, specification of the '031 application at p. 3, lines 3-6.

Claim 48 recites a method wherein calcium is added to the fibrinogen granulate, to the thrombin solution, or to both the fibrinogen granulate and thrombin solution. This claim would have been obvious to one of ordinary skill in the art in light of Count 2 because, as mentioned previously, calcium is a common component of fibrin tissue adhesives. *See*, *e.g.*, the '031 application at p. 2, lines 9-12.

For the foregoing reasons, claims 43-49, and 70-73 would have been obvious in view of Count 2, and, therefore, correspond to this count.

ii. Claims from the '318 patent

Claims 16-19 from the '318 patent correspond to Count 2. As mentioned previously, claims 16-19 of the '318 patent are virtually identical to claims 70-73 of the '031 application, because claims 70-73 of the '031 application were copied from claims 16-19 of the '318 patent.

Similarly to the situation with Count 1, independent claim 16 of the '318 patent also recites methods wherein the granulates have a particle size of 20-1000 µm, whereas Count 2 recites a particle size of 50-1000 µm. As mentioned before, this difference is not sufficient to make the subject matter of claim 16 separately patentable

over Count 2 because of the significant overlap in the ranges. Moreover, this limitation of claim 16 would be met by Count 2 at the end points of the particle size range. Claim 16 also recites that the product temperature does not exceed 50°C. This additional limitation would have been obvious to one of ordinary skill in the art because process temperatures lower than 50°C are normally used to avoid denaturation of the components of a fibrin tissue adhesive. *See, e.g.*, the '318 patent at col. 9, lines 29-25.

Claims 17-19 from the '318 patent are substantially similar to claims 71-73 of the '031 application and would have been obvious in light of Count 2 for the same reasons explained above for claims 71-73 of the '031 application.

For example, claim 17 is drawn to a process wherein separate granules of thrombin and fibrinogen are produced and then mixed. Claim 17 would have been obvious if Count 2 was prior art because preparation of separate formulations of thrombin and fibrinogen is a method well known in the art to store fibrin tissue adhesives. These separate formulations are then mixed at the time when the fibrin adhesive is used. *See, e.g.*, specification of the '031 application at p. 3, lines 3-6.

Claim 18 of the '318 patent recites a process wherein thrombin is sprayed onto fibrinogen dried granules. Claim 18 would have been obvious if Count 2 was prior art because processes wherein two active ingredients are sequentially deposited on a particle are well known in the art. *See, e.g.*, U.S. Patent No. 5,840,329 at Fig. 1d.

Claim 19 is drawn to a process wherein thrombin or fibrinogen solutions or suspensions are sprayed onto a carrier material. Claim 19 would have been obvious if Count 2 was considered prior art because the use of carrier materials is well known in

the art for the preparation of particles comprising active ingredients. *See, e.g.*, U.S. Patent No. 5,840,329 at cols. 1-2.

For the foregoing reasons, claims 16-19 would have been obvious in view of, and therefore correspond to, Count 2.

Appendix I shows the claims in the '031 application containing interfering subject matter as defined in Counts 1 and 2, while Appendix J shows the corresponding claims in the '318 patent containing the interfering subject matter.

VI. Reasons why Applicant will prevail on priority

As required by 37 C.F.R. §§ 41.202(a)(4) and 41.204(d), Applicant submits that Applicant will prevail on priority at least because Applicant can prove a reduction to practice of an embodiment within the scope of Counts 1 and 2 before the earliest possible constructive reduction to practice available to the '318 patent. That is, while not conceding that the '318 patent is entitled to claim benefit of its foreign priority application, Applicant can prove an actual reduction to practice before October 27, 1998, which is the filing date of the '318 patent's German Priority Application (DE 198 49 589).

Appendix C contains documents showing evidence of a reduction to practice of an embodiment falling with the scope of both Counts 1 and 2. One of the documents is a facsimile letter from the inventor of the '031 application, Dr. Mirna Rapp, to a patent attorney forwarding a summary of some results obtained in the development of a fibrin tissue adhesive granulate. The second document is the actual summary of results. The date of the letter, the manual date-stamp when the document was received in the offices of the patent attorney, and the automatic date stamp by the recipient's facsimile

machine have been redacted. However, the undersigned avers that the date of the letter is earlier in time than October 27, 1998, and therefore constitutes evidence of the actual reduction to practice of at least one embodiment within the scope of Counts 1 and 2. Appendix D contains the English-language translation of the documents in Appendix C. Appendix E indicates the location within these documents of the elements of each suggested count.

VII. Claims added or amended to provoke an interference

As required by 37 C.F.R. § 41.202(a)(5), Applicant presents in Appendix F a claim chart showing the location of written description support within the specification for claims added or amended to provoke this interference. Claims 59-65 and 67-73 were copied from the '318 patent. The table in Appendix F shows at least one location in the present specification supporting the copied claims, but does not necessarily include all passages in the specification that support each copied claim.

Claims 59-65 and 67-73 have already been examined by the Office with respect to their compliance with the written description requirement and found to be fully supported by the specification. For example, the Interview Summary mailed March 15, 2006, indicates that the pending claims are allowable. This interview summary was issued after Applicant responded to the Office's initial challenge regarding the sufficiency of the written description support for some of the copied claims in the Office Action dated October 4, 2004.

VIII. Constructive reduction to practice of the proposed counts

37 C.F.R. § 41.202(a)(6) requires that the applicant show, for each constructive reduction to practice for which the applicant wishes to be accorded benefit, where the disclosure provides a constructive reduction to practice within the scope of each count.

The present application claims priority benefit of DE 198 59 611, filed December 23, 1998. Examples 1-3 of DE 198 59 611 (Beispiel 1-3 on p. 4) constitute a constructive reduction to practice of Counts 1 and 2. Examples 1-3 of DE 198 59 611 correspond to Examples 1-3 of the '031 application. The table in Appendix G, shows where in the Examples each element of the count is present in the Examples. The citations are to the Examples in the '031 application, which are in the English language. A copy of DE 198 59 611 is included in Appendix H.

IX. Conclusion

Applicant respectfully requests that an interference be declared using the proposed Counts 1 and 2 set forth in section IV of this paper, with claims 25-28, 37, 52-57, 59-65, and 67-69 from the '031 application and claims 1-15 of the '318 patent corresponding to Count 1; and claims 43-49 and 70-73 from the '031 application and claims 16-19 from the '318 patent corresponding to count 2. Applicant additionally requests that Applicant be accorded the benefit of the filing date of DE 198 59 611 for proposed Counts 1 and 2.

Please grant any extensions of time required to enter this response and charge any additional required fees to our Deposit Account No. 06-0916.

Respectfully submitted,

FINNEGAN, HENDERSON, FARABOW, GARRETT & DUNNER, L.L.P.

By: Reg. No. 41,469
Carlos M. Tellez
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Dated: October 11, 2006

Appendix A

Comparison of claims in the '031 application and the '318 patent

Claims in the '031 application	Claims in the '318 patent
Claim 59 A fibrin tissue adhesive formulation containing a mixture of thrombin, and fibrinogen with factor XIII in flowable solid granules, said mixture prepared by: (a) providing solutions or suspensions of the thrombin, and the fibrinogen with factor XIII; (b) drying the solutions in a fluidized bed apparatus; and (c) forming the flowable solid granules with a particle size of approximately 50-1000 µm. **Claim 25** A fibrin tissue adhesive granulate comprising granulate pellets with a particle size in the range from approximately 50 µm to approximately 1000 µm, wherein said granulate pellets comprise thrombin, Factor XIII, fibrinogen, and a calcium salt.	**Claim 1** A fibrin tissue adhesive formulation containing a mixture of thrombin, and fibrinogen with factor XIII in pourable solid granules, said mixture prepared by: (a) providing solutions or suspensions of the thrombin, and the fibrinogen with factor XIII; (b) drying the solutions in a fluidised bed apparatus; and (c) forming the pourable solid granules with a particle size of 20-1000 µm.

Claims in the '031 application	Claims in the '318 patent
Claim 63 A fibrin tissue adhesive formulation containing a mixture of thrombin, and fibrinogen with factor XIII in flowable solid granules, said mixture prepared by: (a) providing solutions or suspensions of the thrombin, and the fibrinogen with factor XIII; (b) drying the solutions in a fluidized bed apparatus; and (c) forming the flowable solid granules with a particle size of 50-1000 µm; wherein the granules are mixed granules incorporating the fibrinogen in an inner core and the thrombin in an outer layer thereon.	**Claim 5** A fibrin tissue adhesive formulation containing a mixture of thrombin, and fibrinogen with factor XIII in pourable solid granules, said mixture prepared by: (a) providing solutions or suspensions of the thrombin, and the fibrinogen with factor XIII; (b) drying the solutions in a fluidised bed apparatus; and (c) forming the pourable solid granules with a particle size of 20-1000 µm; wherein the granules are mixed granules incorporating the fibrinogen in an inner core and the thrombin in an outer layer thereon.
Claim 64 A fibrin tissue adhesive formulation containing a mixture of thrombin, and fibrinogen with factor XIII in flowable solid granules, said mixture prepared by: (a) providing solutions or suspensions of the thrombin, and the fibrinogen with factor XIII; (b) drying the solutions in a fluidized bed apparatus; and (c) forming the flowable solid granules with a particle size of 50-1000 µm; wherein the mixed granules comprise a carrier core, a fibrinogen layer on the core and an outer thrombin layer.	**Claim 6** A fibrin tissue adhesive formulation containing a mixture of thrombin, and fibrinogen with factor XIII in pourable solid granules, said mixture prepared by: (a) providing solutions or suspensions of the thrombin, and the fibrinogen with factor XIII; (b) drying the solutions in a fluidised bed apparatus; and (c) forming the pourable solid granules with a particle size of 20-1000 µm; wherein the mixed granules comprise a carrier core, a fibrinogen layer on the core and an outer thrombin layer.

Claims in the '031 application	Claims in the '318 patent
Claim 70 A process for producing a fibrin tissue adhesive formulation containing a mixture of thrombin, and fibrinogen with factor XIII in flowable solid granules, which comprises (a) providing solutions or suspensions of the thrombin, and the fibrinogen with factor XIII; (b) drying the solutions in a fluidized bed apparatus; and (c) forming the flowable solid granules, said granules having a particle size of 50-1000 µm. **Claim 43** A method for the preparation of a fibrin tissue adhesive granulate as claimed in claim 25 comprising, suspending the components of the fibrin adhesive in an organic solvent, and spray-drying said suspension to a granulate of particle size in the range from approximately 50 µm to approximately 1000 µm.	**Claim 16** A process for producing a fibrin tissue adhesive formulation containing a mixture of thrombin, and fibrinogen with factor XIII in pourable solid granules, which comprises (a) providing solutions or suspensions of the thrombin, and the fibrinogen with factor XIII; (b) drying the solutions in a fluidised bed apparatus; and (c) forming the pourable solid granules at a product temperature not exceeding 50° C., said granules having a particle size of 20-1000 µm

Appendix B

Claim chart comparing claims from each party to each proposed count

Count 1	Claims in the '031 application	Claims in the '318 patent
	Claim 59	**Claim 1**
A fibrin tissue adhesive formulation containing a mixture of thrombin, and fibrinogen with factor XIII in flowable solid granules, said mixture prepared by: (a) providing solutions or suspensions of the thrombin, and the fibrinogen with factor XIII; (b) drying the solutions in a fluidized bed apparatus; and (c) forming the flowable solid granules with a particle size of approximately 50-1000 µm.	A fibrin tissue adhesive formulation containing a mixture of thrombin, and fibrinogen with factor XIII in flowable solid granules, said mixture prepared by: (a) providing solutions or suspensions of the thrombin, and the fibrinogen with factor XIII; (b) drying the solutions in a fluidized bed apparatus; and (c) forming the flowable solid granules with a particle size of approximately 50-1000 µm.	A fibrin tissue adhesive formulation containing a mixture of thrombin, and fibrinogen with factor XIII in pourable solid granules, said mixture prepared by: (a) providing solutions or suspensions of the thrombin, and the fibrinogen with factor XIII; (b) drying the solutions in a fluidised bed apparatus; and (c) forming the pourable solid granules with a particle size of 20-1000 µm.
Or	**Claim 25**	
A fibrin tissue adhesive granulate comprising granulate pellets with a particle size in the range from approximately 50 µm to approximately 1000 µm, wherein said granulate pellets comprise thrombin, Factor XIII, fibrinogen, and a calcium salt.	A fibrin tissue adhesive granulate comprising granulate pellets with a particle size in the range from approximately 50 µm to approximately 1000 µm, wherein said granulate pellets comprise thrombin, Factor XIII, fibrinogen, and a calcium salt.	

Count 2	Claims in the '031 application	Claims in the '318 patent
A process for producing a fibrin tissue adhesive formulation containing a mixture of thrombin, and fibrinogen with factor XIII in flowable solid granules, which comprises (a) providing solutions or suspensions of the thrombin, and the fibrinogen with factor XIII; (b) drying the solutions in a fluidized bed apparatus; and (c) forming the flowable solid granules, said granules having a particle size of 50-1000 µm. **Or** A method for the preparation of a fibrin tissue adhesive granulate comprising, suspending the components of the fibrin adhesive in an organic solvent, and spray-drying said suspension to a granulate of particle size in the range from approximately 50 µm to approximately 1000 µm wherein the adhesive granulate comprises thrombin, Factor XIII, fibrinogen, and a calcium salt.	**Claim 70** A process for producing a fibrin tissue adhesive formulation containing a mixture of thrombin, and fibrinogen with factor XIII in flowable solid granules, which comprises (a) providing solutions or suspensions of the thrombin, and the fibrinogen with factor XIII; (b) drying the solutions in a fluidized bed apparatus; and (c) forming the flowable solid granules, said granules having a particle size of 50-1000 µm. **Claim 43** A method for the preparation of a fibrin tissue adhesive granulate as claimed in claim 25 comprising, suspending the components of the fibrin adhesive in an organic solvent, and spray-drying said suspension to a granulate of particle size in the range from approximately 50 µm to approximately 1000 µm.	**Claim 16** A process for producing a fibrin tissue adhesive formulation containing a mixture of thrombin, and fibrinogen with factor XIII in pourable solid granules, which comprises (a) providing solutions or suspensions of the thrombin, and the fibrinogen with factor XIII; (b) drying the solutions in a fluidised bed apparatus; and (c) forming the pourable solid granules at a product temperature not exceeding 50° C., said granules having a particle size of 20-1000 µm

Appendix C

Documents showing reduction to practice of

an embodiment within Counts 1 and 2

REDACTED

Anlage 22

Fax

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CENTEON
Ein Unternehmen von Aventis und Behring

An/To: Dr. K.-H. Meyer-Dulheuer C9721
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Von/From: Dr. Mirna Rapp

REDACTED

Datum/Date:

Sehr geehrter Herr Dr. Meyer-Dulheuer,

beiliegend schicke ich Ihnen eine kurze Zusammenfassung über unsere in Zusammenarbeit mit der Firma Glatt GmbH durchgeführte Entwicklung eines Einkomponenten-Fibrinklebers in Pulverform.
Mit der Fa. Glatt wurde bereits darüber gesprochen, unsere bisherigen Ergebnisse als ein gemeinsames Patent anzumelden, bei dem Centeon den biochemischen Teil und Glatt den technischen Prozess beschreibt.

Leider liegt bereits eine Patentanmeldung der Fa. Andaris Ltd. über die Sprühtrocknung von Fibrinogen und Thrombin vor.

Aus meiner Sicht besteht ein deutlicher Unterschied bei unserem Produkt in den physikalischen Eigenschaften der Partikel, wie Granulat-Form, Größe (>60 µm), Staubfreiheit, Rieselfähigkeit und gute Löslichkeit. Außerdem haben wir bereits die Machbarkeit eines Pulvers gezeigt, der alle Fibrinkleberkomponenten in einem Partikel enthält.

Hiermit möchte ich Sie bitten, die begefügten Unterlagen zu prüfen und mir mitzuteilen, ob und worin die Möglichkeit einer Patentanmeldung von unserer Seite besteht. Dann werde ich einen Entwurf für die Patentanmeldung verfassen und ihn Ihnen so bald wie möglich zukommen lassen. Sollten Sie noch offene Fragen haben oder zusätzliches Informationsmaterial benötigen, so wenden Sie sich bitte jederzeit an mich.

Vielen Dank für Ihre Bemühungen und ich verbleibe

mit freundlichen Grüßen

M. Rapp

(Dr. M. Rapp)

Anlagen:

1. Entwicklungsstand unseres Fibrinkleber-Granulats
2. Patentanmeldung der Fa. Andaris (WO 97/44015)
3. Patentanmeldung der Fa. Glatt GmbH (WO 96/15849)

Entwicklung eines Fibrinkleber-Granulats in Zusammenarbeit mit der Firma Glatt GmbH

Unser Ziel:

Entwicklung eines rieselfähigen, nicht staubenden und gut löslichen Pulvers, welches die Fibrinkleber-Komponenten Fibrinogen, Thrombin und F XIII zusammen in einem Partikel enthält.
Beim Auftragen auf die Wunde, d.h. durch Kontakt mit einem wässrigen Medium reagieren die o.g. Komponenten zu einem Fibringel, der zum Wundverschluss führt.

Vorteile eines solchen Pulvers:

- Einfachere Handhabung als der herkömmliche Fibrinkleber Beriplast™, da keine Vorbereitungen notwendig sind, sozusagen ein gebrauchsfertiges Produkt (Anwendung imNotfall); Auftragen auf die Wundfläche nach Art eines „Salzstreuers"
- Lagerung bei 2-8°C sowie bei Raumtemperatur wird angestrebt
- Gute Dosierungsmöglichkeit, z.B. bei Hauttransplantationen

Stand unserer Entwicklung:

1. Versuchsserie

Bei der Firma Glatt GmbH wurde unser Fibrinogenkonzentrat (enthält neben Fibrinogen Humanserumalbumin, Fibronektin, Aminosäuren und Salze) aus 10%iger wässriger Proteinlösung in Wirbelschicht nach dem Top-Spray-Verfahren sprühgetrocknet.

Dabei wurden zwei Varianten erprobt:

1) Sprühtrocknung von Fibrinogen auf Mannitol als Vorlage (Mannitol könnte die Löslichkeit des relativ schwerlöslichen Fibrinogens verbessern)
2) Sprühtrocknung von Fibrinogen alleine, d.h. ohne Träger

Die apparativen Sprühtrocknungsbedingungen, wie Temperatur des Fluidisationsgases (in unserem Falle Luft), Sprühdruck etc. wurden soweit optimiert, daß keine Denaturierung des

Proteins auftritt und eine durchschnittliche Partikelgröße von 100 µm resultiert. Ist die Partikelgröße kleiner, dann ist das Pulver nicht mehr staubfrei.

Bei dem o.g. Verfahren entstehen keine kompakten Partikel wie z.B. kleine Kügelchen, sondern ein Granulat mit „Kanälen" (körnige Beschaffenheit). Dadurch erreicht man eine relativ große Partkelgröße, womit das Produkt staubfrei und gut löslich wird (ähnlich wie beim Instant-Kaffee!).

2. Versuchsserie:

Nachdem die analytischen Untersuchungen in unserem Labor gezeigt haben, daß die Aktivität von Fibrinogen und FXIII nach der Sprühtrocknung voll wiederfindbar ist, wurde die Herstellung von Granulat versucht, welches alle nötigen Fibrinkleberkomponenten in einem Partikel enthält.

- Sprühtrocknung von Fibrinogen(konzentrat) aus wässriger Lösung auf Mannitol als Vorlage
- Auf das erhaltene Fibrinogen/Mannitol-Granulat wurden Thrombin/$CaCl_2$ aus isopropanolischer Suspension aufgesprüht. Das organische Lösungsmittel Isopropanol verhindert die Bildung von Fibrin nach Kontakt von Fibrinogen mit dem Thrombin.

Wir erhielten ein rieselfähiges, nicht staubendes Pulver mit einer durchschnittlichen Granulatgröße von 100 µm. Dieses Fibrinkleber-Granulat läßt sich wie Salz auf eine Wasseroberfläche aufstreuen, wo es sofort ein Fibringel bildet.
Genaue analytische Untersuchungen bezüglich der Aktivität der einzelnen Komponenten werden zur Zeit in unserem Labor durchgeführt. Biomechanische Untersuchungen sind ab Mitte dieser Woche geplant.

Es wurde auch versucht, eine isopropanolische Suspension von Fibrinogen sprühzutrocknen, mit dem Fernziel, das Fibrinkleber-Granulat in einem Schritt, d.h. alle Komponenten aus einer Suspension heraus, zu versprühen. Leider ist das nicht machbar, da die Fibrinogensuspension auch nach Utraschallbehandlung zu grobe Partikel enthält, die die Sprühtrocknungdüse verstopfen. Auch haben wir gezeigt, daß eine Zerkleinerung der Partikel im Ultraturrax eine Inaktivierung von Fibrinogen zur Folge hat. Die Thrombin-Isopropanol-Suspension ist viel feiner und damit sprühfähig.

Geplante Versuche:

- Herstellung eines Fibrinkleber-Granulats ohne Mannitol
- Das Fibrinogen-Granulat wird mit einer bioverträglichen Inertschicht (z.B. PVP) überzogen, auf die dann Thrombin aus wässriger Lösung aufgesprüht wird
- In vivo-Machbarkeit am Tiermodell

Appendix D

English-language translation of the documents from Appendix C

(Documents showing reduction to practice of

an embodiment within Counts 1 and 2)

[Stamp: **REDACTED**] Attachment 22

CENTEON
An Armour and Behring Company

Fax

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Fax No.:	069 5 97 50 59		
From:	Dr. Mirna Rapp		
Date:	**REDACTED**		

Dear Dr. Meyer-Dulheuer,

Attached, please find a brief summary concerning our development of a single component fibrin adhesive in powder form, which was performed in cooperation with Glatt GmbH.
We have already discussed with Glatt to file a joint patent for the results we have achieved so far; Centeon would be describing the biochemical portion and Glatt would be describing the technical process.

Unfortunately, Andaris Ltd has already filed a patent application on the spray drying of fibrinogen and thrombin.

In my opinion, our product is clearly different with respect to the physical properties of the particles such as the form of the granules, the size (> 60 µm), no dusting, pourability and good solubility. Furthermore, we have already demonstrated the feasibility of a powder that comprises all fibrin adhesive components in one particle.

This is to kindly request you to review the attached documentation and letting me know if we could file a patent application and what we could claim. I will then draft a patent application and forward it to you as soon as possible. Please contact me any time if you have further questions or require additional information.

With many thanks for your efforts, I remain

With best regards,

[*Signed*]

(Dr. M. Rapp)

Enclosures:

1. Status of the development of our fibrin adhesive granulate
2. Patent application filed by Andaris (WO 97/44015)
3. Patent application filed by Glatt GmbH (WO 96/15849)

Development of a fibrin adhesive granulate in cooperation with Glatt GmbH

Our goal:

To develop a pourable, dust-free powder that has good solubility and comprises the fibrin components fibrinogen, thrombin and F XIII in one particle.

When applied to the wound, i.e. through contact with an aqueous medium, the aforementioned components will react into a fibrin gel which causes the wound to close.

Advantages of a powder of this type:

- Easier to handle than the conventional fibrin adhesive Beriplast^{TM} because it does not require any preparations, i.e., it is a ready-to-use product (application in emergencies); it can be applied to the surface area of the wound similar to a "salt shaker"
- We strive for storage at 2-8 °Celsius as well as at room temperature
- Good dosing options, such as in skin transplantation

Status of our development:

1st Testing series

Our fibrinogen concentrate (which in addition to fibrinogen also contains human serum albumen, fibronectin, amino acids and salts) was spray dried from 10-% aqueous protein solution in the fluidized bed according to the top spray method at Glatt GmbH.

Two variants were tested:

1) Spray drying of fibrinogen using mannitol as receiver (mannitol may improve the solubility of the fibrinogen, which is relatively poor)
2) Spray drying of fibrinogen alone, i.e., without a carrier

The spray drying conditions of the apparatus, such as the temperature of the fluidization gas (which, in our case, was air), the spraying pressure, etc. were optimized to the extent that no denaturation of the protein is experienced and an average particle size of 100 µm is obtained. If the particle size is smaller, the powder is no longer dust-free.

The aforementioned method does not yield any compact particles such as small pellets, for example, but rather granules with "ducts" (grainy consistency). This yields a relatively large particle size, which makes the product dust-free and give it good solubility (similar to instant coffee!).

2nd Testing series

After the analytical experiments in our laboratories revealed that the activity of fibrinogen and FX III can be completely recovered after spray drying, we attempted to produce granules that contain all required fibrin adhesive components in one particle.

- Spray drying of fibrinogen (concentrate) from aqueous solution on mannitol as receiver

- Thrombin / $CaCl_2$ from an isopropanol suspension were sprayed on the fibrinogen / mannitol granule product. The organic solvent isopropanol prevents the formation of fibrin after the fibrinogen comes into contact with thrombin.

We obtained a pourable, dust-free powder with an average granule size of 100 µm. These fibrin adhesive granules can be sprinkled like salt onto an aqueous surface area, where they immediately form a fibrin gel.
Specific analytical experiments with respect to the activity of the individual components are currently being performed in our laboratory. Bio-mechanical experiments are scheduled to start mid-week.

We also attempted to spray-dry an isopropanolic suspension of fibrinogen with the ultimate goal to spray the fibrin adhesive granules in one step, i.e., all components from one suspension. Unfortunately, that is not doable because even after ultrasound treatment, the fibrinogen suspension contains particles that are too large and clog the spray drying nozzle. We also demonstrated that reducing the size of the particles in the Ultraturrax leads to an inactivation of fibrinogen. The thrombin-isopropanol suspension is much smaller and thus suitable for spraying.

Scheduled experiments:

- Production of fibrin adhesive granules without mannitol
- Coating the fibrinogen granules with a bio-compatible inert layer (such as PVP, for example) and then spraying thrombin from aqueous solution on said inert layer
- *In vivo* feasibility on the animal model.

Appendix E

Actual reduction to practice of embodiments within the suggested counts

Count 1	Citation to the English translation of the document (Appendix D)
A fibrin tissue adhesive formulation containing…	"These fibrin adhesive granules can be sprinkled like salt onto an aqueous surface area." Page 5, lines 4-6.
…a mixture of thrombin, and fibrinogen with factor XIII…	"After the analytical experiments in our laboratories revealed that the activity of fibrinogen and F XIII can be completely recovered after spray drying, we attempted to produce granules that contain all required fibrin adhesive components in one particle." Page 4, lines 14-16. "Thrombin/$CaCl_2$ from an isopropanol suspension were sprayed on the fibrinogen/mannitol granule product." Page 5, lines 1-2.
…in flowable solid granules, said mixture prepared by:	"We obtained a pourable, dust-free powder." Page 5, line 4.
(a) providing solutions or suspensions of the thrombin, and the fibrinogen with factor XIII;	"Thrombin/$CaCl_2$ from an isopropanol suspension were sprayed on the fibrinogen/mannitol granule product." Page 5, lines 1-2.

Count 1	Citation to the English translation of the document (Appendix D)
(b) drying the solutions in a fluidized bed apparatus; and	"The spray drying conditions of the apparatus, such as the temperature of the fluidization gas (which, in our case, was air), the spraying pressure, etc. were optimized to the extent that no denaturation of the protein is experienced." Page 4, lines 5-7.
(c) forming the flowable solid granules with a particle size of approximately 50-1000 µm.	"We obtained a pourable, dust-free powder with an average granule size of 100 µm." Page 5, line 4.
Or	
A fibrin tissue adhesive granulate comprising granulate pellets with a particle size in the range from approximately 50 µm to approximately 1000 µm, wherein said granulate pellets comprise thrombin, Factor XIII, fibrinogen, and a calcium salt.	"After the analytical experiments in our laboratories revealed that the activity of fibrinogen and F XIII can be completely recovered after spray drying, we attempted to produce granules that contain all required fibrin adhesive components in one particle." Page 4, lines 14-16. "Thrombin/$CaCl_2$ from an isopropanol suspension were sprayed on the fibrinogen/mannitol granule product. We obtained a pourable, dust-free powder with an average granule size of 100 µm." Page 5, lines 1-4.

Count 2	Citation to the English translation of the document (Appendix D)
A process for producing a fibrin tissue adhesive formulation containing a mixture of thrombin, and fibrinogen with factor XIII...	"After the analytical experiments in our laboratories revealed that the activity of fibrinogen and F XIII can be completely recovered after spray drying, we attempted to produce granules that contain all required fibrin adhesive components in one particle." Page 4, lines 14-16.
...in flowable solid granules, which comprises...	"We obtained a pourable, dust-free powder." Page 5, line 4.
(a) providing solutions or suspensions of the thrombin, and the fibrinogen with factor XIII;	"Thrombin/$CaCl_2$ from an isopropanol suspension were sprayed on the fibrinogen/mannitol granule product." Page 5, lines 1-2.
(b) drying the solutions in a fluidized bed apparatus; and	"The spray drying conditions of the apparatus, such as the temperature of the fluidization gas (which, in our case, was air), the spraying pressure, etc. were optimized to the extent that no denaturation of the protein is experienced." Page 4, lines 5-7.
(c) forming the flowable solid granules, said granules having a particle size of 50-1000 μm.	"We obtained a pourable, dust-free powder with an average granule size of 100 μm." Page 5, line 4.

Count 2	Citation to the English translation of the document (Appendix D)
Or A method for the preparation of a fibrin tissue adhesive granulate comprising, suspending the components of the fibrin adhesive in an organic solvent, and spray-drying said suspension to a granulate of particle size in the range from approximately 50 µm to approximately 1000 µm wherein the adhesive granulate comprises thrombin, Factor XIII, fibrinogen, and a calcium salt.	"After the analytical experiments in our laboratories revealed that the activity of fibrinogen and F XIII can be completely recovered after spray drying, we attempted to produce granules that contain all required fibrin adhesive components in one particle." Page 4, lines 14-16. "Thrombin/$CaCl_2$ from an isopropanol suspension were sprayed on the fibrinogen/mannitol granule product. We obtained a pourable, dust-free powder with an average granule size of 100 µm." Page 5, lines 1-4.

Appendix F

Claim chart showing written description support for claims added or amended to provoke an interference.

Claims	Support in Specification
59	p. 4, line 3 to p. 6, line 2
60	p. 7, lines 12-20
61	p. 6, lines 3-9; p. 7, lines 3-10; Examples 1, 2, & 3
62	p. 6, lines 3-9; p.10, line 23 to p.11, line 9
63	p. 7, lines 3-8; Examples 1 & 3
64	Examples 2 & 3
65	p. 7, lines 12-20
67	p. 4, lines 13-17
68	p. 11, lines 10-29; Examples 6-8
69	p. 4, lines 13-17
70	p. 4, line 30 to p. 6, line 2; Examples 1-4
71	p. 7, lines 12-20; Example 4
72	Examples 2 & 3
73	p. 6, lines 3-9; p. 7, lines 3-10; Examples 1-3

Appendix G

Constructive reduction to practice of embodiments within the suggested counts

Count 1	Citation is to Examples 1-4 in the '031 application, which correspond to Beispiel 1-4 on p. 4 of DE 198 59 611
A fibrin tissue adhesive formulation containing...	"Preparation of fibrin adhesive granulate." Title of Example 3, at p. 14, line 21.
...a mixture of thrombin, and fibrinogen with factor XIII...	An isopropanolic thrombin/$CaCl_2$-suspension was sprayed on the fibrinogen granulate prepared in Examples 1 or 2. Example 3, at p. 14, lines 22-23. "A 10 % protein solution of Beriplast®-fibrinogen concentrate (also contains F XIII). was spray dried according to the top-spray-method in a fluidized bed." Example 1, at p. 13, lines 21-23.
...in flowable solid granules, said mixture prepared by:	"The fibrin adhesive granulate prepared in this manner had a mean particle size of 100 µm; [and] it was flowable." Example 3, at p. 14, lines 17-18.
(a) providing solutions or suspensions of the thrombin, and the fibrinogen with factor XIII; (b) drying the solutions in a fluidized bed apparatus; and	An isopropanolic thrombin/$CaCl_2$-suspension was sprayed on the fibrinogen granulate prepared in Examples 1 or 2. Example 3, at p. 14, lines 22-23. "A 10 % protein solution of Beriplast®-fibrinogen concentrate (also contains F XIII). was spray dried according to the top-spray-method in a fluidized bed." Example 1, at p. 13, lines 21-23.

Count 1	Citation is to Examples 1-4 in the '031 application, which correspond to Beispiel 1-4 on p. 4 of DE 198 59 611
(c) forming the flowable solid granules with a particle size of approximately 50-1000 µm.	"The fibrin adhesive granulate prepared in this manner had a mean particle size of 100 µm; [and] it was flowable." Example 3, at p. 14, lines 17-18.
Or A fibrin tissue adhesive granulate comprising granulate pellets	"Preparation of fibrin adhesive granulate." Title of Example 3, at p. 14, line 21.
with a particle size in the range from approximately 50 µm to approximately 1000 µm,	"The fibrin adhesive granulate prepared in this manner had a mean particle size of 100 µm; [and] it was flowable." Example 3, at p. 14, lines 17-18.
wherein said granulate pellets comprise thrombin, Factor XIII, fibrinogen, and a calcium salt.	An isopropanolic thrombin/$CaCl_2$-suspension was sprayed on the fibrinogen granulate prepared in Examples 1 or 2. Example 3, at p. 14, lines 22-23. "A 10 % protein solution of Beriplast®-fibrinogen concentrate (also contains F XIII). was spray dried according to the top-spray-method in a fluidized bed." Example 1, at p. 13, lines 21-23.

Count 2	Citation is to Examples 1-3 in the '031 application, which correspond to Beispiel 1-3 on p. 4 of DE 198 59 611
A process for producing a fibrin tissue adhesive formulation...	"Preparation of fibrin adhesive granulate." Title of Example 3, at p. 14, line 21.
...containing a mixture of thrombin, and fibrinogen with factor XIII...	An isopropanolic thrombin/$CaCl_2$-suspension was sprayed on the fibrinogen granulate prepared in Examples 1 or 2. Example 3, at p. 14, lines 22-23. "A 10 % protein solution of Beriplast®-fibrinogen concentrate (also contains F XIII). was spray dried according to the top-spray-method in a fluidized bed." Example 1, at p. 13, lines 21-23.
...in flowable solid granules, which comprises...	"The fibrin adhesive granulate prepared in this manner had a mean particle size of 100 µm; [and] it was flowable." Example 3, at p. 14, lines 17-18.
(a) providing solutions or suspensions of the thrombin, and the fibrinogen with factor XIII; (b) drying the solutions in a fluidized bed apparatus; and	An isopropanolic thrombin/$CaCl_2$-suspension was sprayed on the fibrinogen granulate prepared in Examples 1 or 2. Example 3, at p. 14, lines 22-23. "A 10 % protein solution of Beriplast®-fibrinogen concentrate (also contains F XIII). was spray dried according to the top-spray-method in a fluidized bed." Example 1, at p. 13, lines 21-23.
	"The fibrin adhesive granulate prepared in this

Count 2	Citation is to Examples 1-3 in the '031 application, which correspond to Beispiel 1-3 on p. 4 of DE 198 59 611
(c) forming the flowable solid granules, said granules having a particle size of 50-1000 µm. **Or** A method for the preparation of a fibrin tissue adhesive granulate comprising, suspending the components of the fibrin adhesive in an organic solvent, and spray-drying said suspension to a granulate of particle size in the range from approximately 50 µm to approximately 1000 µm wherein the adhesive granulate comprises thrombin, Factor XIII, fibrinogen, and a calcium salt.	manner had a mean particle size of 100 µm; [and] it was flowable." Example 3, at p. 14, lines 17-18. "Preparation of fibrin adhesive granulate." Title of Example 3, at p. 14, line 21. An isopropanolic thrombin/$CaCl_2$-suspension was sprayed on the fibrinogen granulate prepared in Examples 1 or 2. Example 3, at p. 14, lines 22-23. "The fibrin adhesive granulate prepared in this manner had a mean particle size of 100 µm; [and] it was flowable." Example 3, at p. 14, lines 17-18. An isopropanolic thrombin/$CaCl_2$-suspension was sprayed on the fibrinogen granulate prepared in Examples 1 or 2. Example 3, at p. 14, lines 22-23.

Appendix H

Copy of German Patent Application No. DE 198 59 611

(19) BUNDESREPUBLIK DEUTSCHLAND

DEUTSCHES PATENT- UND MARKENAMT

(12) **Offenlegungsschrift**

(10) **DE 198 59 611 A 1**

(51) Int. Cl.[7]: A 61 K 38/36

(21) Aktenzeichen: 198 59 611.1
(22) Anmeldetag: 23. 12. 1998
(43) Offenlegungstag: 29. 6. 2000

(71) Anmelder:
Centeon Pharma GmbH, 35037 Marburg, DE

(72) Erfinder:
Rapp, Mirna, Dr., 35037 Marburg, DE

Die folgenden Angaben sind den vom Anmelder eingereichten Unterlagen entnommen

Prüfungsantrag gem. § 44 PatG ist gestellt

(54) Fibrinklebergranulat und Verfahren zu dessen Herstellung

(57) Es wird ein rieselfähiges Fibrinklebegranulat beschrieben, das Thrombin, Faktor XIII, Fibrinogen und ein Kalziumsalz in Granulatkörnchen mit einer Partikelgröße von über 50 µm bis 1000 µm enthält. Ein derartiges Granulat wird in einem evakuierbaren Behälter mittels eines Fluidisationsgases in der Wirbelschicht durch Sprühtrocknung hergestellt. Es eignet sich zur Wundheilung in der Chirurgie, der Gewebstherapie und/oder als Trägermaterial für biologische Faktoren.

DE 198 59 611 A 1

DE 198 59 611 A 1

Beschreibung

Gegenstand der Erfindung ist ein rieselfähiges Fibrinklebergranulat, das alle zur Bildung eines stabilen Fibringels erforderlichen Substanzen enthält und direkt zur Wundverklebung eingesetzt werden kann. Es wird durch die Sprühtrocknung in der Wirbelschicht mittels eines Fluidisationsgases erzeugt.

Es ist bekannt, daß nach der Entstehung einer Wunde die Wundheilung durch eine Aktivierungskaskade mehrerer hintereinandergeschalteter Gerinnungsfaktoren eingeleitet wird. Daraus resultiert am Ende die Reaktion zwischen dem aktivierten Thrombin und Fibrinogen in Gegenwart von Kalziumionen zur Bildung einer Fibrinmatrix, die schließlich die Wunde abdeckt und somit eine Hämostase bewirkt. Diese Fibrinmatrix wird durch den aktivierten Faktor XIII (F XIIIa) über zusätzliche kovalente Bindungen weiter verfestigt, wodurch deren mechanische Stabilität und Resistenz gegen einen vorzeitigen proteolytischen Abbau erhöht wird.

In der modernen Chirurgie gewinnt die Hämostase durch Fibrinklebung zunehmend an Bedeutung, da es sich bei dem sogenannten Fibrinkleber um ein gut verträgliches und die Wundheilung förderndes Biomaterial handelt. Diese Methode eignet sich hervorragend zur Hämostase von stark blutenden Wunden bei Operationen an parenchymatösen, inneren Organen, bei Hauttransplantationen, in der Notfallchirurgie bei inneren und äußeren Verletzungen, vor allem aber auch zur unterstützenden Abdichtung von Nähten zur Vermeidung postoperativer Blutungen. In der HNO- und Gesichts-Chirurgie wird für die Heilung äußerer Wunden der Fibrinkleber dem Nahtverschluß aus kosmetischen Gründen vorgezogen. Auch wird der Fibrinkleber zunehmend in der endoskopischen Chirurgie z. B. zur Blutstillung von Magengeschwüren eingesetzt.

Die heute im Handel befindlichen Fibrinkleber wie Beriplast® enthalten neben anorganischen Salzen und Aminosäuren, die aus dem Humanplasma gewonnenen Gerinnungsfaktoren Fibrinogen, Thrombin und Faktor XIII, zusätzlich auch Albumin und Fibronektin zur Förderung der Wundheilung. Obwohl das Präparat sehr gute biochemische und hämostatische Eigenschaften aufweist, bedarf es einer aufwendigen Vorbereitung vor seiner Anwendung. Die voneinander getrennten Fibrinogen- und Thrombinlyophilisate werden separat aufgelöst, in zwei voneinander getrennten Spritzen aufgezogen und in eine spezielle Halterung/Vorrichtung eingespannt. Dieses Verfahren erfordert neben viel Zeit auch gut geschultes Personal. Eine Variante des Fibrinklebers wird als Tissucol® wird bereits in fertig gelöster Form in Spritzen in den Handel gebracht, ist jedoch nur bei tiefen Temperaturen von –20°C lagerfähig und muß vor der Anwendung im Wasserbad aufgetaut werden. Somit finden beide Varianten des Fibrinklebers keine Anwendung in Situationen, wo ein gebrauchsfertiger und sofort ohne Vorbereitungen einsetzbarer Fibrinkleber von Nöten ist. Außerdem wäre ein gebrauchsfertiger und gut dosierbarer Fibrinkleber auch deshalb kostengünstiger, weil überschüssiges Material weder unnötig vorbereitet noch verworfen zu werden braucht.

Eine mögliche Verbesserung der Handhabung des Fibrinklebers könnte ein Einkomponentenkleber sein, der alle zur Bildung des Fibrins nötigen Komponenten in einem Kompartiment beinhaltet. Die Entwicklung eines Einkomponentenklebers in einer wässrigen Lösung ist allerdings in der Praxis sehr schwer realisierbar. Eine Möglichkeit besteht allenfalls darin, die Komponenten des Fibrinklebers im getrockneten Zustand zu mischen, womit sich diese nach Applikation auf der Wunde in der Blutflüssigkeit oder dem Wundexsudat lösen und in situ eine Fibrinmatrix ausbilden, die eine Hämostase bewirkt. Dafür ist auch notwendig, das naturgemäß schwer lösliche Fibrinogen in eine derartige trockene Form zu bringen, aus der es rasch in Lösung geht und dabei sofort mit dem Thrombin reagiert.

Es hat auch schon Versuche gegeben, mit einem gezielten Lyophilisationsverfahren Partikel zu entwickeln, die Fibrinogen oder Thrombin enthalten und nach der Herstellung miteinander vermischt auf der Wunde aktiviert werden. So wird in der Internationalen Patentanmeldung WO 97/44015 die Herstellung sogenannter Mikropartikel auf Fibrinogen- oder Thrombinbasis beschrieben, die jeweils einzeln sprühgetrocknet werden. Diese Mikropartikel bestehen zu über 90% aus Körnchen mit einer Größe bis zum 20 µm. Sie sollen gut löslich sein und können miteinander vermischt zur Wundheilung eingesetzt werden. Ein Nachteil dieser Mikropartikel ist allerdings, daß es sich dabei um ein sehr stark staubendes Pulver handelt, wodurch eine direkte Applikation wie das Aufstreuen auf eine Wunde nicht möglich ist. Ein derartiges Pulver benötigt daher ein spezielles Applikationssystem, was seine Handhabung und klinische Indikationen drastisch einschränkt.

Es stellte sich deshalb die Aufgabe, ein Fibrinklebergranulat zu entwickeln, das gut löslich, rieselfähig und nicht staubend ist und damit direkt auf die Wunde aufgestreut werden kann z. B. nach dem Prinzip eines Salzstreuers.

Diese Aufgabe wird erfindungsgemäß durch ein rieselfähiges Fibrinklebergranulat gelöst, das Thrombin, Faktor XIII, Fibrinogen und ein Kalziumsalz in Granulatkörnchen mit einer Partikelgröße von über 50 bis etwa 1000 µm, vorzugsweise mit einer Partikelgröße von 100 bis 200 µm enthält. Aufgrund dieser Partikelgröße ist das erfindungsgemäße Fibrinklebergranulat nicht staubend, gut löslich und rieselfähig und kann hervorragend auf eine Wundfläche oder ein feuchtes Gewebe aufgetragen werden, wo es sofort eine Fibrinmatrix ausbildet.

Einem derartigen Fibrinklebergranulat können auch noch Albumin, Fibronektin, Aminosäuren und physiologisch verträgliche anorganische Salze zugesetzt werden. Es kann außerdem als ein Freisetzungssystem für biologische, pflanzliche und/oder synthetische Faktoren verwendet werden. Diese Faktoren können die Wundheilung unterstützen oder als Antifibrinolytika, Antibiotika, Chemotherapeutika oder Immunmodulatoren wirken. Sie werden dem Fibrinklebergranulat während des Sprühtrocknungsprozesses zugesetzt.

Ein geeignetes Verfahrensprinzip für die Herstellung des erfindungsgemäßen Fibrinklebergranulats ist bereits aus der Internationalen Patentanmeldung WO 96/15849 bekannt. Dort ist ein Verfahren zur Trocknung von Blutplasma, Blutplasmafraktionen oder daraus gewonnen Blutplasmaprodukten beschrieben, bei dem das Behandlungsgut im flüssigen oder gelösten Zustand in einen evakuierbaren Behälter gesprüht wird, wodurch die Trocknung – bis zur Granulatform – mittels eines Fluidisationsgases in der Wirbelschicht durchgeführt wird. Auf Fibrinogen und Thrombin kann dieses Verfahren jedoch nicht ohne weiteres angewendet werden, da diese Substanzen bekannterweise nach Kontakt mit wässrigen Lösungen zu Fibrin reagieren. Deshalb kommt die Anwendung wässriger Lösungen für die Sprühtrocknung dieser Komponenten nicht in Frage. Um trotzdem beide Komponenten in einem Partikel zu erhalten, werden die Komponenten erfindungsgemäß in einem organischen Lösungsmittel suspendiert und daraus sprühgetrocknet. Fibrinogen, Thrombin und

Faktor XIII lassen sich in organischen Lösungsmitteln wie den niederen Alkoholen, Aceton, Nitrilen, flüssigen Karbonsäureestern, Ethern, Chloroform, Dimethylformamid und Dimethylsulfoxid mehr oder weniger homogen suspendieren, ohne daß sie eine Reaktion zu Fibrin zeigen. Nach Entfernung des organischen Lösungsmittels sind sie in wässriger Phase wieder zur Fibrinbildung befähigt.

Erfindungsgemäß erfolgt die Sprühtrocknung dabei entweder durch das Top-Spray-Verfahren, bei dem die Flüssigkeit im Gegenstrom zum Fluidisationsverfahren geführt wird oder im Gleichstrom (Bottom-Spray-Verfahren). Durch das Einsprühen des flüssigen Behandlungsgutes in den evakuierbaren Behälter durch eine geeignete Düse wird eine feine Verteilung erreicht. Das Fluidisationsgas dient dabei sowohl zur Verwirbelung des zu behandelnden Gutes als auch zur Wärmeübertragung. Deshalb wird ein erwärmtes Gas als Fluidisationsgas eingesetzt. Durch die Messung der Produkttemperatur während des Wirbelschichtprozesses und einer darauf basierenden Prozeßsteuerung kann eine produktschonende Trocknung eingehalten werden. Als Fluidisationsgas kann entweder Luft oder ein Inertgas wie Stickstoff eingesetzt werden. Die Trocknung wird dabei so lange fortgeführt, bis das Behandlungsgut in fein verteilter Granulatform und einer Partikelgröße von 50 bis etwa 1000 µm, vorzugsweise von 100 bis 200 µm vorliegt.

Das erfindungsgemäße Fibrinklebergranulat kann mit oder ohne ein in den evakuierbaren Behälter vorgelegtes Trägermaterial hergestellt werden. Als Trägermaterial kommen dabei vor allem Zucker und Zuckeralkohole wie Sacharose, Lactose oder Mannit in Betracht, die gut bioverträglich sind.

Ein besonders bevorzugtes Verfahren besteht in einer zweistufigen Sprühtrocknung, bei der zuerst ein Fibrinogengranulat hergestellt wird. Dieses Granulat kann neben Fibrinogen auch andere Proteine, Kohlenhydrate, Aminosäuren und physiologisch verträglich anorganische Salze enthalten. Die Partikelgröße dieses Granulates beträgt mehr als 50 bis etwa 1000 µm, bevorzugt wird jedoch eine Partikelgröße von 70 bis 200 µm. Auf dieses Fibrinogengranulat wird eine feine Thrombin-Suspension in einem organischen Lösungsmittel aufgesprüht, die bereits gelöste Kalziumionen enthält. Die Konzentration der Kalziumionen beträgt 1 bis 100 mM, vorzugsweise 10 bis 50 mM. Man erhält auf diese Weise ein Fibrinklebergranulat mit einer Partikelgröße, die vorzugsweise zwischen 100 und 200 µm liegt und eine körnige, sehr gut lösliche Struktur aufweist. Dabei entstehen keine kompakten Partikel wie kleine Kügelchen, sondern ein Granulat mit vielen feinen Kanälen. Dadurch erreicht man eine relativ große Partikelgröße, wodurch das Produkt gleichzeitig staubfrei und gut löslich wird, ähnlich wie der bekannte Instant-Kaffee. Dieses Granulat läßt sich hervorragend auf eine Wundfläche aufstreuen und bildet nach Kontakt mit einem wässrigen Medium sofort ein festes und elastisches Fibringel.

Das erfindungsgemäße Fibrinklebergranulat ist jedoch auch durch Sprühtrocknung von Fibrinogen-Konzentrat aus einer wässrigen Lösung auf eine Vorlage z. B. Mannit, erhältlich. Dabei wird zunächst ein Fibrinogen/Mannit-Granulat erhalten, auf das dann anschließend Thrombin/Kalziumsalz, z. B. aus isopropanolischer Suspension, aufgesprüht wird. Das organische Lösungsmittel verhindert die Bildung von Fibrin nach Kontakt von Fibrinogen mit dem Thrombin.

Schließlich ist es aber auch möglich, separat Fibrinogen- und Thrombin-Granulate mit der vorstehend genannten Partikelgröße in getrennten Verfahren herzustellen, wobei beide Substanzen aus wässrigen Lösungen sprühgetrocknet werden können. Allerdings benötigt man dann bei der Herstellung des Thrombingranulates einen ausreichenden Anteil an Trägermaterial, da im Fibrinkleber die Menge an Thrombin gewichtsmäßig um den Faktor 102 bis 103 kleiner ist als diejenige von Fibrinogen. Diese beiden Granulate werden miteinander vermischt und können dann entsprechend zur Hämostase und Wundheilung eingesetzt werden.

Die nach den vorstehend genannten Verfahren hergestellten Fibrinklebergranulate wurden anschließend auf ihre biomechanischen Eigenschaften untersucht und dabei die folgenden Ergebnisse erzielt:

Reisskraft nach in vitro Hautklebung (Klebefläche: 2,25 cm^2)

Ergebnis einer Vergleichsstudie anhand einer Randomisierungsliste zu der Reisskraft des einheitlichen Granulates (Thrombin, Fibrinogen und Faktor XIII in einem Partikel), des Granulat-Gemisches (Fibrinogen-Granulat + Thrombin-Granulat) und des flüssigen Fibrinklebers (Beriplast®):

Testsubstanz	Reisskraft
Einheitliches Granulat	3,3 N
Granulat-Gemisch	1,8 N
Beriplast®	1,5 N

Die gemessenen Werte zeigen deutlich den Vorteil des einheitlichen Granulates gegenüber dem Granulatgemisch bezüglich der biomechanischen Eigenschaften. Die Menge an aktiven Komponenten war in allen drei Testsubstanzen annähern identisch.

Das erfindungsgemäße rieselfähige Fibrinklebergranulat unterscheidet sich durch eine einfachere Handhabung von den bisher bekannten Fibrinklebern, da keine Vorbereitungsmaßnahmen notwendig sind und es sich stets im gebrauchsfertigen Zustand befindet. Es ist deshalb ganz besonders für die Notfallchirurgie geeignet. Es hat außerdem den Vorteil der außerordentlich einfachen Anwendung durch Auftragen auf die Wundflächen nach der Art eines Salzstreuers. Es eignet sich hervorragend für chirurgische Anwendungen, bei denen eine rasche Hämostase durch Aufsaugen von Blut und gleichzeitige Fibrinklebung erreicht werden sollen.

Die Erfindung wird durch die nachfolgenden Beispiele erläutert.

Beispiel 1

Herstellung von Fibrinogen-Granulat ohne Vorlage

Eine 10%ige Proteinlösung von Beriplast®-Fibrinogenkonzentrat (enthält auch F XIII) wurde nach dem Top-Spray-Verfahren in Wirbelschicht sprühgetrocknet. Dieses Verfahren wurde in einer GPCG 1-Anlage der Firma Glatt GmbH durchgeführt und ist in der Internationalen Patentanmeldung WO 96/15849 beansprucht und detailliert beschrieben. Die Bedingungen sind:
Eingangstemperatur: 37°C
Ausgangstemperatur: 30°C
Sprühdruck: 3,0 bar
Sprührate: 3,2 g/min
Das derart hergestellte Fibrinogen-Granulat hatte eine mittlere Partikelgröße von 100 µm und war sehr gut löslich. Analytische Aktivitätsmessungen haben gezeigt, daß die Aktivität von Fibrinogen und F XIII durch den Sprühtrocknungsprozeß bei diesen Bedingungen nicht beeinträchtigt wird.

Beispiel 2

Herstellung von Fibrinogen-Granulaten mit Vorlage

200 g Mannit oder Albumin wurden in die Sprühtrocknungskammer vorgelegt. Auf die Vorlage wurde 100 g Fibrinogenkonzentrat in Wirbelschicht unter folgenden Bedingung aufgesprüht:
Eingangstemperatur: 30°C
Ausgangstemperatur: 24°C
Sprühdruck: 2,5 bar
Sprührate: 3,0–8,0 g/min
Es resultierte ein rieselfähiges und gut lösliches Granulat mit einer mittleren Partikelgröße von 100 µm, bei dem die Fibrinogen- und F XIII-Aktivität voll wiederfindbar ist.

Beispiel 3

Herstellung von Fibrinkleber-Granulat

Auf das in Beispiel 1 oder 2 hergestellte Fibrinogen-Granulat wurde eine isopropanolische Thrombin/$CaCl_2$-Suspension aufgesprüht. Der Prozeß lief unter folgenden Bedingungen ab:
Eingangstemperatur: 30°C
Ausgangstemperatur: 25°C
Sprühdruck: 2,5 bar
Sprührate: 3,0–8,0 g/min
Das auf diese Weise hergestellte Fibrinkleber-Granulat mit einer mittleren Partikelgröße von 100 µm war rieselfähig, staubt nicht und bildete sofort nach Kontakt mit einer wässrigen Lösung ein stabiles, d. h. durch F XIII kovalent vernetztes Fibringerinnsel.

Beispiel 4

Herstellung von Thrombin-Granulat

Auf eine Vorlage von Mannit oder Humanserumalbumin wurde eine wässrige 0,3%ige Thrombin-Lösung aufgesprüht. Die Bedingungen waren wie folgt:
Eingangstemperatur: 30°C
Ausgangstemperatur: 23°C
Sprühdruck: 2,5 bar
Sprührate: 4,2 g/min
Die mittlere Partikelgröße des gebildeten Granulates betrug ca. 65 µm. Es war rieselfähig und nicht staubend. Es ließ sich gut mit dem Fibrinogen-Granulat mischen und war auch als Fibrinkleber einsetzbar.

Patentansprüche

1. Rieselfähiges Fibrinkleber-Granulat, **dadurch gekennzeichnet**, daß es Granulatkörnchen mit einer Partikelgröße von über 50 bis etwa 1000 µm enthält, die Thrombin, Faktor XIII, Fibrinogen und ein Kalziumsalz enthalten.
2. Fibrinkleber-Granulat nach Anspruch 1, dadurch gekennzeichnet, daß die Granulat-Körnchen eine Partikelgröße von 100 bis 200 µm aufweisen.
3. Fibrinkleber-Granulat nach den Ansprüchen 1 und 2, dadurch gekennzeichnet, daß es Albumin, Fibronektin und/oder weitere die Wundheilung fördernde Substanzen enthält.
4. Verfahren zur Herstellung des Fibrinkleber-Granulates der Ansprüche 1 bis 3, dadurch gekennzeichnet, daß ein oder mehrere Bestandteile des Fibrinklebers in einem organischen Lösungsmittel suspendiert und in einem evakuierbaren Behälter mittels eines Fluidisationsgases in der Wirbelschicht bis zu einer Partikelgröße von über 50 bis 1000 µm, vorzugsweise 100 bis 200 µm, sprühgetrocknet werden, wobei anschließend die Granulatkörnchen gege-

benfalls noch miteinander vermischt werden können.

5. Verfahren nach Anspruch 4, dadurch gekennzeichnet, daß es mit oder ohne ein in den Behälter vorgelegtes Trägermaterial hergestellt wird.

6. Verfahren nach den Ansprüchen 4 und 5, dadurch gekennzeichnet, daß zunächst ein Fibrinogen-Granulat hergestellt wird, auf welches eine Suspension eines organischen Lösungsmittels enthaltend Thrombin und ein Kalziumsalz aufgesprüht wird.

7. Verfahren zur Herstellung eines Fibrinkleber-Granulates nach den Ansprüchen 1 bis 3, dadurch gekennzeichnet, daß die separat hergestellten Fibrinogen- und Thrombin-Granulatkörnchen, die jeweils eine Partikelgröße von über 50 µm bis etwa 1000 µm aufweisen, miteinander vermischt werden.

8. Verwendung eines Fibrinkleber-Granulates nach den Ansprüchen 1 bis 3, dadurch gekennzeichnet, daß es zur Wundheilung in der Chirurgie, der Gewebstherapie und/oder als Trägermaterial für biologische Faktoren eingesetzt wird.

- Leerseite -

Appendix I

Claims in the '031 application corresponding to Counts 1 and 2

25. (Previously presented) A fibrin adhesive granulate comprising granulate pellets with a particle size in the range from approximately 50 µm to approximately 1000 µm, wherein said granulate pellets comprise thrombin, Factor XIII, fibrinogen, and a calcium salt.

26. (Previously presented) The fibrin adhesive granulate in accordance with claim 25, wherein the granulate pellets have a particle size in the range from approximately 100 µm to approximately 200 µm.

27. (Previously presented) The fibrin adhesive granulate in accordance with claim 25, wherein said granulate pellets further comprise one or more substances chosen from albumin, fibronectin, and other substances that promote wound healing.

28. (Previously presented) The fibrin adhesive granulate in accordance with claim 26, wherein said granulate pellets further comprise one or more substances chosen from albumin, fibronectin, and other substances that promote wound healing.

37. (Previously presented) A preparation comprising a fibrin adhesive granulate as claimed in any one of claims 25 or 27.

43. (Previously presented) A method for the preparation of a fibrin adhesive granulate as claimed in claim 25 comprising,
suspending the components of the fibrin adhesive in an organic solvent, and spray-drying said suspension to a granulate of particle size in the range from approximately 50 µm to approximately 1000 µm.

44. (Previously presented) The method in accordance with claim 43, wherein the particle size of the granulate is in the range from approximately 100 µm to approximately 200 µm.

45. (Previously presented) The method in accordance with claim 43, wherein the suspension is spray-dried onto a support medium.

46. (Previously presented) The method in accordance with claim 44, wherein the suspension is spray-dried onto a support medium.

47. (Previously presented) A method for the preparation of a fibrin adhesive granulate as claimed in claim 25, comprising
preparing a fibrinogen granulate, and
spraying an organic solvent comprising thrombin onto said fibrinogen granulate.

48. (Currently Amended) The method in accordance with claim 47, wherein a calcium salt is added to the fibrinogen granulate, to the thrombin ~~solution~~ suspension, or to both the fibrinogen granulate and the thrombin ~~solution~~suspension.

49. (Previously presented) A method for the preparation of a fibrin adhesive granulate as claimed in claim 25, comprising
preparing separate fibrinogen and thrombin granulates, and
mixing the fibrinogen granulates with the thrombin granulates,
wherein both types of granulates have a particle size in the range from approximately 50 µm to approximately 1000 µm.

52. (Previously presented) A method for preparing a preparation comprising adding one or more biological, vegetable or synthetic active substances to the fibrin adhesive granulate as claimed in claim 25.

53. (Previously presented) The method in accordance with claim 52, wherein said one or more biological, vegetable or synthetic active substances are chosen from immunoglobulins, chemotherapeutics and antibiotics.

54. (Previously presented) A method for achieving hemostasis comprising applying a fibrin adhesive preparation to an area in need thereof, wherein the fibrin

adhesive preparation comprises a fibrin adhesive granulate as claimed in claim 25.

55. (Previously presented) A method for healing a wound in surgery comprising applying a fibrin adhesive preparation to an area in need thereof, wherein the fibrin adhesive preparation comprises a fibrin adhesive granulate as claimed in claim 25.

56. (Previously presented) A method for effecting tissue therapy comprising applying a fibrin adhesive preparation to an area in need thereof, wherein the fibrin adhesive preparation comprises a fibrin adhesive granulate as claimed in claim 25.

57. (Previously presented) A method for preparing a support medium for one or more biological, vegetable or synthetic factors comprising mixing said one or more biological, vegetable or synthetic factors with a fibrin adhesive preparation, wherein the fibrin adhesive preparation comprises a fibrin adhesive granulate as claimed in claim 25.

59. (Previously presented) A fibrin tissue adhesive formulation containing a mixture of thrombin, and fibrinogen with factor XIII in flowable solid granules, said mixture prepared by:

(a) providing solutions or suspensions of the thrombin, and the fibrinogen with factor XIII;

(b) drying the solutions in a fluidized bed apparatus; and

(c) forming the flowable solid granules with a particle size of approximately 50-1000 µm.

60. (Previously presented) The fibrin tissue adhesive formulation of claim 59, wherein the thrombin and fibrinogen granules have been separately dried.

61. (Previously presented) The fibrin tissue adhesive formulation of claim 59, wherein the thrombin and/or fibrinogen granules have a support medium as carrier.

62. (Previously presented) The fibrin tissue adhesive formulation of claim 61, wherein the support medium is selected from sugars, sugar alcohols, proteins, and mixtures thereof.

63. (Previously presented) A fibrin tissue adhesive formulation containing a mixture of thrombin, and fibrinogen with factor XIII in flowable solid granules, said mixture prepared by:

(a) providing solutions or suspensions of the thrombin, and the fibrinogen with factor XIII;

(b) drying the solutions in a fluidized bed apparatus; and

(c) forming the flowable solid granules with a particle size of 50-1000 µm; wherein the granules are mixed granules incorporating the fibrinogen in an inner core and the thrombin in an outer layer thereon.

64. (Previously presented) A fibrin tissue adhesive formulation containing a mixture of thrombin, and fibrinogen with factor XIII in flowable solid granules, said mixture prepared by:

(a) providing solutions or suspensions of the thrombin, and the fibrinogen with factor XIII;

(b) drying the solutions in a fluidized bed apparatus; and

(c) forming the flowable solid granules with a particle size of 50-1000 µm; wherein the mixed granules comprise a carrier core, a fibrinogen layer on the core and an outer thrombin layer.

65. (Previously presented) The fibrin tissue adhesive formulation of claim 59, wherein the ratio of thrombin to fibrinogen with factor XIII is 1:100 to 1:1000.

67. (Previously presented) The fibrin tissue adhesive formulation of claim 59, wherein the grain diameter of the granules is 100-200 µm.

68. (Previously presented) The fibrin tissue adhesive formulation of claim 59, wherein the granules are covered with an outer barrier layer.

69. (Previously presented) The fibrin tissue adhesive formulation of claim 59, wherein the solution or suspension contains a calcium salt.

70. (Previously presented) A process for producing a fibrin tissue adhesive formulation containing a mixture of thrombin, and fibrinogen with factor XIII in flowable solid granules, which comprises

(a) providing solutions or suspensions of the thrombin, and the fibrinogen with factor XIII;

(b) drying the solutions in a fluidized bed apparatus; and

(c) forming the flowable solid granules, said granules having a particle size of 50-1000 μm.

71. (Previously presented) The process of claim 70, wherein:

(a) a fibrinogen concentrate with factor XIII is sprayed into the fluidized bed apparatus from aqueous solution, dried and isolated;

(b) a thrombin concentrate is sprayed into the fluidized bed apparatus from aqueous solution, dried and isolated; and

(c) the granules of fibrinogen and thrombin thus produced are mixed.

72. (Previously presented) The process for producing a fibrin tissue adhesive formulation of claim 70, wherein;

(a) fibrinogen concentrate is sprayed into the fluidized bed apparatus from aqueous solution and dried; and

(b) thrombin is sprayed onto the dried granules from an organic suspension.

73. (Previously presented) The process for producing a fibrin tissue adhesive formulation of claim 70, wherein the solutions or suspensions are sprayed onto a carrier material.

Appendix J

Claims in the '318 patent corresponding to Counts 1 and 2

1. A fibrin tissue adhesive formulation containing a mixture of thrombin, and fibrinogen with factor XIII in pourable solid granules, said mixture prepared by:

(a) providing solutions or suspensions of the thrombin, and the fibrinogen with factor XIII;

(b) drying the solutions in a fluidised bed apparatus; and

(c) forming the pourable solid granules with a particle size of 20-1000 μm.

2. The fibrin tissue adhesive formulation of claim 1, wherein the mixture consists of separately dried thrombin and fibrinogen granules.

3. The fibrin tissue adhesive formulation of claim 1, wherein the thrombin and/or fibrinogen granules have a core as carrier.

4. The fibrin tissue adhesive formulation of claim 3, wherein the carrier is selected from water-soluble sugars, sugar substitutes, biological transport substances, or mixtures thereof.

5. A fibrin tissue adhesive formulation containing a mixture of thrombin, and fibrinogen with factor XIII in pourable solid granules, said mixture prepared by:

(a) providing solutions or suspensions of the thrombin, and the fibrinogen with factor XIII;

(b) drying the solutions in a fluidised bed apparatus; and

(c) forming the pourable solid granules with a particle size of 20-1000 μm;

wherein the granules are mixed granules incorporating the fibrinogen in an inner core and the thrombin in an outer layer thereon.

6. A fibrin tissue adhesive formulation containing a mixture of thrombin, and fibrinogen with factor XIII in pourable solid granules, said mixture prepared by:

(a) providing solutions or suspensions of the thrombin, and the fibrinogen with factor XIII;

(b) drying the solutions in a fluidised bed apparatus; and

(c) forming the pourable solid granules with a particle size of 20-1000 μm;

wherein the mixed granules comprise a carrier core, a fibrinogen layer on the core and an outer thrombin layer.

7. The fibrin tissue adhesive formulation of claim 5 or 6, wherein a barrier layer is present between the fibrinogen layer and the outer thrombin layer.

8. The fibrin tissue adhesive formulation of claim 7, wherein the barrier layer is produced by drying solutions of low-molecular polyvinylpyrrolidone; cellulose derivatives; or carbohydrates.

9. The fibrin tissue adhesive formulation of claim 1, wherein the ratio of thrombin to fibrinogen with factor XIII is 1:10 to 1:1000.

10. The fibrin tissue adhesive formulation of claim 9, wherein the ratio of thrombin to fibrinogen with factor XIII is 1:50 to 1:200.

11. The fibrin tissue adhesive formulation of claim 1, wherein the grain diameter of the granules is 30-500 µm.

12. The fibrin tissue adhesive formulation of claim 11, wherein the grain diameter of the granules is 40-200 µm.

13. The fibrin tissue adhesive formulation of claim 1, wherein the granules are provided with an outer barrier layer.

14. The fibrin tissue adhesive formulation of claim 1, wherein thrombin and fibrinogen are produced recombinantly by genetic engineering or biotechnological processes.

15. The fibrin tissue adhesive formulation of claim 1, wherein the solution or suspension contains a calcium salt.

16. A process for producing a fibrin tissue adhesive formulation containing a mixture of thrombin, and fibrinogen with factor XIII in pourable solid granules, which comprises

(a) providing solutions or suspensions of the thrombin, and the fibrinogen with factor XIII;

(b) drying the solutions in a fluidised bed apparatus; and

(c) forming the pourable solid granules at a product temperature not exceeding 50°C., said granules having a particle size of 20-1000 µm.

17. The process of claim 16, wherein:

(a) a fibrinogen concentrate with factor XIII is sprayed into the fluidised bed apparatus from aqueous solution, dried and isolated;

(b) a thrombin concentrate is sprayed into the fluidised bed apparatus from aqueous solution, dried and isolated; and

(c) the granules of fibrinogen and thrombin thus produced are mixed.

18. The process for producing a fibrin tissue adhesive formulation of claim 16, wherein:

(a) fibrinogen concentrate is sprayed into the fluidised bed apparatus from aqueous solution and dried; and

(b) thrombin is sprayed onto the dried granules from an organic suspension.

19. The process for producing a fibrin tissue adhesive formulation of claim 16, wherein the solutions or suspensions are sprayed onto a carrier material.